Biochem. J. (1976) **155**, 567–570
Printed in Great Britain

A New Method for Deriving Steady-State Rate Equations Suitable for Manual or Computer Use

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(*Received* 7 *November* 1975)

A schematic method for the derivation of steady-state enzyme rate equations by using the Wang algebra is described. The method is simple, easy to learn and offers a substantial decrease in analytical effort over previously published algorithms. Being essentially an algebraic procedure the method can be readily computerized. Computer programs in BASIC and ALGOL languages have been deposited as Supplementary Publication SUP 50065 (19 pages) at the British Library (Lending Division), Boston Spa, Wetherby, W. Yorkshire LS23 7BQ, U.K., from whom copies can be obtained on the terms indicated in *Biochem. J.* (1976), **153**, 5.

The existence of a number of schematic methods for the derivation of steady-state rate equations for enzyme mechanisms (King & Altman, 1956; Volkenstein & Goldstein, 1966; Fisher & Schulze, 1969; Fromm, 1970; Orsi, 1972; Seshagiri, 1972) reflects the interest of enzymologists in the solution of steady-state kinetic problems. The schematic method of King & Altman (1956) is used widely, although it is well known that their procedure becomes unwieldy with complex mechanisms (Orsi, 1972; Volkenstein & Goldstein, 1966). These difficulties inherent in the King & Altman (1956) algorithm are largely circumvented by generating the valid King & Altman (1956) patterns systematically by using the method of Lam & Priest (1972), but the procedure remains laborious, particularly when the mechanism contains irreversible steps. In such cases only a relatively small fraction of the valid King & Altman (1956) patterns are permissible combinations of reaction steps.

Topological graph theory is the basis of the algorithm used by Volkenstein & Goldstein (1966). Their method is more efficient than that of King & Altman (1956), but in some respects it is difficult to apply (Fromm, 1970) and requires considerable practice.

A simple new algorithm is presented here which has the merits of being purely algebraic, and as such is easy to program for a digital computer. Moreover, apart from the unavoidable tedium, complex mechanisms are solved manually as readily as are simple kinetic mechanisms. In this method we generate the denominator of the rate equation, from which the appropriate numerator terms are then selected.

Algorithm Description

The new algorithm can be considered as an extension of the Lam & Priest (1972) method. Lam & Priest (1972) represent the enzyme mechanism as a non-oriented connected linear graph, from which they generate the valid King & Altman (1956) patterns, or trees by applying the Wang algebra (Duffin, 1959) and alphanumeric multiplication. Here we apply similar manipulations, but to a directed graph (Seshu & Reed, 1961), i.e. to the enzyme mechanism as written.

The Wang algebra states that the sum or product of identical constants is equal to zero.

$$x \cdot x = 0 \quad \text{for all } x$$
$$x + x = 0 \quad \text{for all } x$$

Alphanumeric multiplication is simply a concatenation of the elements multiplied, e.g.

$$(12+34)(56+78) = 1256+1278+3456+3478$$

The following steps are used in deriving the steady-state rate equation.

1. Write down the reaction mechanism including all rate constants and their directions in graphical form.
2. Circle $(n-1)$ nodes (i.e. enzyme forms), where n is the number of nodes in the mechanism. The result is independent of the node that is omitted. (However, selection of the node omitted can simplify the solution by eliminating the possibility of certain cycles arising.)
3. List separately the rate constants cut by each of the $(n-1)$ circles.

4. List also all combinations of pairs of rate constants which leave each individual node of the graph. These are forbidden combinations of rate constants.

5. By using the Wang algebra and eliminating the forbidden combinations listed in step 4, multiply alphanumerically the listing obtained in step 3.

6. The product from step 5 is the denominator of the rate equation which is sorted into nodes, i.e. expressions or determinants for each enzyme form.

7. Node sorting, e.g. for enzyme form EX_i, is accomplished by selecting those terms in the denominator expression that do not contain rate constants directed away from EX_i.

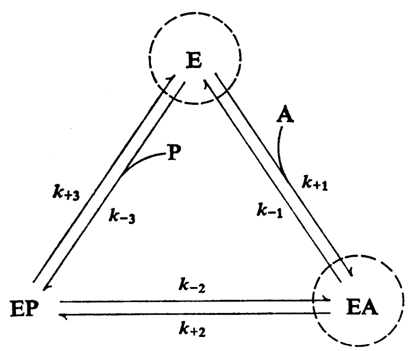
8. Assemble the rate equation in the usual way (King & Altman, 1956; Volkenstein & Goldstein, 1966).

When expanding the steady-state solution in step 5 it is convenient to manipulate only the subscripts of the rate constants, and to omit any substrate or product concentrations associated with particular rate constants. These are readily inserted later (step 8) to complete the solution.

Two further shorthand devices aid the calculation. One is to separate the elements of the arrays by commas, it being understood that the completed denominator expression is the sum of these elements. A second device is to express rate constants of the form k_{-x} as x'. Combinations such as xx' would be eliminated from the solution by the Wang summation rule, since they represent cycles, but such expressions are more readily recognized and discarded during step 5 if this convention is used, i.e. $xx'=0$ for all x.

Application of the algorithm is illustrated in detail by the solution of the trivial Uni Uni mechanism $E+A \rightleftharpoons EA \rightleftharpoons EP \rightleftharpoons E+P$ shown in Scheme 1. In Scheme 2 a more complex schematic mechanism, representing for example a random Uni Bi reaction or a random-substrate-addition, ordered-product-release Bi Bi reaction, is shown. The solution is not developed beyond step 5, although those terms which constitute the determinant of node 2 (not 2 or 1′) are underlined as an example of node sorting. Scheme 2 demonstrates the relative simplicity of the

Scheme 1. *The algorithm as applied to a Uni Uni mechanism*



Step 4 Forbidden combinations
13′; 1′2; 2′3

Step 3 (1, 1′, 3, 3′)(1, 1′, 2, 2′)

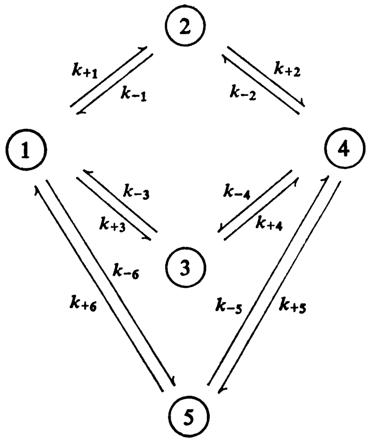
Step 5 Multiplier (exclusions)	Product
1 (3′)	12, 12′
1′(2)	1′2′
3 (2′)	13, 1′3, 23
3′(1)	1′3′, 23′, 2′3′
Step 7 Node selection	
Node E (not 1 or 3′)	1′2′, 1′3, 23
Node EA (not 1′ or 2)	12′, 13, 2′3′
Node EP (not 2′ or 3)	12, 1′3′, 23′

$$\text{Rate } v=\frac{(k_{+3}[EP]-k_{-3}[E][P])[E_0]}{[E]+[EA]+[EP]}$$

$$=\frac{(k_{+1}k_{+2}k_{+3}[A]-k_{-1}k_{-2}k_{-3}[P])[E_0]}{k_{-1}k_{-2}+k_{-1}k_{+3}+k_{+2}k_{+3}+[A](k_{+1}k_{-2}+k_{+1}k_{+3}+k_{+1}k_{+2})+[P](k_{-2}k_{-3}+k_{-1}k_{-3}+k_{+2}k_{-3})}$$

where $[E_0]=[E]+[EA]+[EP]$

Scheme 2. *Application of the algorithm to a schematic mechanism*



Forbidden combinations

13
1′2
2′4′
4′5
2′5
3′4
16′
36′
5′6

Step 3 Omitting node 1 (see text)
(2, 2′, 4, 4′, 5, 5′) (1, 1′, 2, 2′) (3, 3′, 4, 4′) (5, 5′, 6, 6′)

Step 5 M = multiplier, relevant exclusions in parentheses
P = product after Wang algebra

M	P	M	P
2 (1′)	12,	3 (1)	1′2′3, 1′34, 234, 2′34, 1′34′, 234′, 1′35,
2′	12′, 1′2′,		235, 1′35′, 235′, 2′35′,
4	14, 1′4, 24, 2′4,	3′(4)	123′, 12′3′, 1′2′3′, 13′4′, 1′3′4′, 23′4′, 13′5,
4′(2′)	14′, 1′4′, 24′,		1′3′5, 23′5, 13′5′, 1′3′5′, 23′5′, 2′3′5′,
5 (2′)	15, 1′5, 25,	4	124, 12′4, 1′2′4, 145, 1′45, 245, 145′,
5′	15′, 1′5′, 25′, 2′5′		1′45′, 245′, 2′45′,
		4′(2′, 5)	124′, 14′5′, 1′4′5′, 24′5′

M	P
5 (2′, 4′)	1′345, 2345, 123′5, 1245,
5′	1′2′35′, 1′345′, 2345′, 2′345′, 1′34′5′, 234′5′, 123′5′, 12′3′5′,
	1′2′3′5′, 13′4′5′, 1′3′4′5′, 23′4′5′, 1245′, 12′45′, 1′2′45′, 124′5′,
6 (5′)	1′2′36, 1′346, 2346, 2′346, 1′34′6, 234′6, 1′356, 2356, 123′6,
	12′3′6, 1′2′3′6, 13′4′6, 1′3′4′6, 23′4′6, 13′56, 1′3′56, 23′56,
	1246, 12′46, 1′2′46, 1456, 1′456, 2456, 124′6,
6′(1, 3)	1′2′3′6′, 1′3′4′6′, 23′4′6′, 1′3′56′, 23′56′, 1′3′5′6′, 23′5′6′, 2′3′5′6′,
	1′2′46′, 1′456′, 2456′, 1′45′6′, 245′6′, 2′45′6′, 1′4′5′6′, 24′5′6′

method and shows a convenient way of setting out the calculation.

It is an important point that by choosing to omit node 1 in the calculation the possibility of cycles arising is eliminated. If node 5 had been omitted instead, then the cycles 123′4′ and 1′2′34 each appear twice in the solution. It is a property of the algorithm that such combinations of rate constants (cycles) are always duplicated in the solution and are thus eliminated by the Wang algebra summation rule. This property is also exploited in the computer program to eliminate invalid cycles. With more complex mechanisms it may be impossible to avoid generating invalid cycles by an appropriate choice of the omitted node. Inspection of the problem, however, shows where these will first occur and in a manual solution they can be eliminated at that stage. If this is not done then invalid cyclic terms will be present in the final denominator expression from step 5. In these cases we recommend that node sorting is first carried out, since the duplicated terms of a particular cycle both segregate to one node, where they are more readily identified.

Discussion

The method has been tested on some 20 kinetic mechanisms containing up to nine nodes and 32 rate constants. These were mechanisms consisting of linear unbranching chains of reaction steps with from three to eight nodes, and random or branching mechanisms. The latter included the seven random mechanisms detailed by Plowman (1972), the random Bi Bi mechanism, the two mnemonical enzyme models of Ricard *et al.* (1974), the bivalent-1 carrier mechanism (Wong, 1965) and the peroxidase mechanism proposed by Childs & Bardsley (1975). In addition, a number of mechanisms representing kinetic formulations of the allosteric models of Adair–Koshland–Némethy–Filmer (Koshland *et al.*, 1966) and of Monod–Wyman–Changeux (Monod *et al.*, 1965) studied by W. G. Bardsley & R. D. Waight (unpublished work) were examined.

Except for the peroxidase mechanism, the rate equations derived either manually or by computer agreed with published solutions or with equations derived by the Volkenstein & Goldstein (1966) method or by the King & Altman (1956) algorithm. Four of the solutions given by Plowman (1972) contain minor errors. The rate equation for the proposed mechanism of horseradish peroxidase (Childs & Bardsley, 1975) was found to be incorrect. The denominator of the rate equation given by them contains only 301 terms of the total 386 terms which comprise the correct solution. The necessary corrections are detailed in Supplementary Publication SUP 50065.

A rigid proof of the method such as that given by Duffin (1959) and by Maxwell & Cline (1966) of the Wang algebra procedure with non-oriented linear graphs is not at present available.

A computer program which carries out the algorithm presented here has been developed. BASIC and ALGOL versions of the program as used on Data General Corporation Nova 820 (with RDOS disk system) computers have been deposited as Supplementary Publication SUP 50065 at the British Library (Lending Division), Boston Spa, Wetherby, W. Yorkshire LS23 7BQ, U.K. The deposited material includes examples of the use of the computer programs.

We thank Professor B. R. Pullan and Dr. R. W. Foster, Medical School, University of Manchester, for the use of the computers. R. E. C. thanks the Medical Research Council for financial support.

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